AWARD NUMBER: W81XWH-17-1-0589

TITLE: **Targeting Diet-Microbiome Interactions in the Pathogenesis of Parkinson's Disease**

PRINCIPAL INVESTIGATOR: Sarkis K. Mazmanian, PhD

CONTRACTING ORGANIZATION: California Institute of Technology
Pasadena, CA 91125

REPORT DATE: September 2018

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT: Approved for Public Release;
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REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

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1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED
Sept 2018	Annual	1 Sep 2017 - 31 Aug 2018

4. TITLE AND SUBTITLE

Targeting Diet-Microbiome Interactions in the Pathogenesis of Parkinson's Disease

5a. CONTRACT NUMBER

5b. GRANT NUMBER
W81XWH-17-1-0589

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
Sarkis K. Mazmanian, Ph.D

E-Mail: sarkis@caltech.edu

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

California Institute of Technology

1200 E California Bl.
Mailcode: 156-29
Pasadena CA 91125

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING AGENCY NAME AND ADDRESS.

U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT

Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT

The current project will analyze the gut microbiome and metabolites from PD patients and controls, and employ clinically relevant mouse models to determine how metabolites produced by the microbiome from dietary substrates affect motor symptoms. We propose to test whether directly regulating microbial metabolite profiles using "designer" dietary fibers and probiotics offers new avenues for ameliorating PD-like symptoms.

15. SUBJECT TERMS

Parkinson's disease, human subjects, intestinal microbiome, stool specimens, gut-brain axis

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			USAMRMC
Unclassified	Unclassified	Unclassified	Unclassified	11	19b. TELEPHONE NUMBER *(include area code)*

Standard Form 298 (Rev. 8-98)
Prescribed by ANSI Std. Z39.18

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INTRODUCTION: The current project will analyze the gut microbiome and metabolites from PD patients and controls, and employ clinically relevant mouse models to determine how metabolites produced by the microbiome from dietary substrates affect motor symptoms. We propose to test whether directly regulating microbial metabolite profiles using "designer" dietary fibers and probiotics offers new avenues for ameliorating PD-like symptoms. During this reporting period 16 new human subjects (100% of targeted enrollment) were successfully recruited at RUMC site. We have advanced the objectives of the project either on time, or in some cases ahead of schedule. The project has, to date, not experienced any major setbacks.

1. **KEYWORDS:** *Parkinson's disease, human subjects, intestinal microbiome, stool specimens, gut-brain axis, intestinal bacteria, dietary fiber, short chain fatty acids*
2. **ACCOMPLISHMENTS:**

- **What were the major goals of the project?**

Major Task 1: Recruitment and Microbiome Sequencing

Subtask 1- subject recruitment and sample collection.	*12 month target of 16 human subjects with stool and tissue collection successfully recruited.*	*100% completed*
Subtask 2- microbiome sequencing / metagenomics.	*24 month timeline.*	*0% completed*
Subtask 3- SCFA analysis for stool and serum.	*12 month timeline.*	*20% completed*

Major Task 2: Animal colonization and phenotyping

Subtask 1 – colonization of mice with human microbiota	*36 month timeline.*	*50% completed*
Subtask 2 – microbiome profiling.	*36 month timeline.*	*0% completed*
Subtask 2 – motor testing, neuroinflammation status.	*36 month timeline.*	*75% completed*
Subtask 3 – AAV cloning and injection.	*6 month timeline.*	*90% completed*
Subtask 4 – CLARITY analysis and electrophysiology.	*36 month timeline.*	*0% completed*

Major Task 3: Fiber testing and treatment of animals

Subtask 1 – treat PD mice with fibers and motor tests.	*12 month timeline.*	*100% completed*
Subtask 2 – treat PD mice with "optimized" fibers & test	*36 month timeline.*	*0% completed*

- **What was accomplished under these goals?**

Activities accomplished in this quarter include: 1) reached our 12 month goal for recruitment, with the target of 16 subject already recruited; 2) colonization of germ-free WT and ASO mice with human microbiota; 3) SCFA treatment of SPF mice followed by motor testing; 4) feeding of SCFAs to SPF mice and analysis of neuroinflammation; 5) production and treatment of animals with prebiotic fibers, and 5 motor testing mice fed prebiotic fibers. We are excited to report that the first round of SCFA feeding to SPF animals showed an effect on motor symptoms. Namely, feeding designer prebiotic diets enriched in 20% butyrate or acetate each improved motor symptoms in mice, whereas the 20% propionate diet did not

have this effect, showing specificity for different SCFAs in our mouse model of PD. Further, we show that butyrate reduces activation of microglia in vitro, and thus may affect neuroinflammation in vivo. There have been no setbacks or failure to achieve a goal, and the project is progressing on the proposed timeline or in some cases such as the microglia studies, ahead of schedule.

Figure legend: As shows by the sticker test, and in the other motor tests, butyrate and acetate improve motor performance while propionate has no effect.

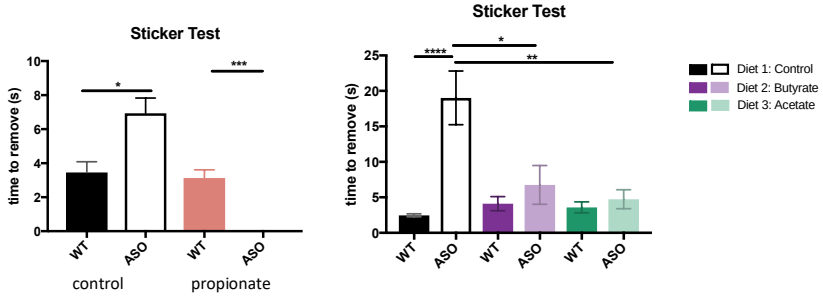
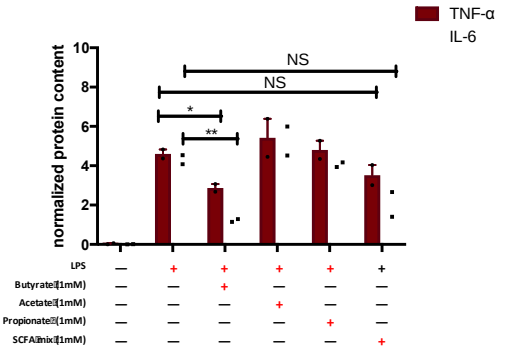


Figure legend: Primary mouse microglia, cultured in vitro with LPS for activation, shows reduced TNF and IL-6 production with butyrate treatment.



- **What opportunities for training and professional development has the project provided?**

Research. *Trainees meet weekly with the PI, both separately and together, to discuss their latest results, technical problems, collaborations, reagent needs and so forth.*

Group Meeting & Scientific discussions. *The Mazmanian laboratory holds formal 2 meetings every week, one for research updates from investigators and the other to discuss literature. Each week a group member presents their work. Often, we have PIs, students and fellows from other laboratories join in our weekly meetings. Each trainee presents their work approximately every 4 weeks to the entire group. These lab meetings cover a range of topics, from immunology, neuroscience, behavior to microbiology to animal models of disease. We frequently discuss relevant papers in the field and how they impact the research in our laboratory. Furthermore, each trainee participates in Caltech's vigorous seminar program in which outside scientists come to Caltech to present their research. They also have the opportunity to participate in the weekly "BioLunch", which features two half-hour*

presentations every week by a student and/or postdoc, thereby providing excellent exposure to ongoing projects in the Biology Division. Further, the 2 postdoctoral fellows will present their work once a year in a campus-wide seminar series called "Micro Mornings", where members of the microbiology community at Caltech discuss their work in front of an audience of peers that include not only biologists, but chemists and engineers as well. The diverse feedback from this worthwhile helps students and fellows craft dynamic research programs. In addition, the students and fellows in the laboratory organize their own weekly journal club, practice talks and brainstorming sessions, often without me.

***Mentoring**. The PI mentors each trainee on science, their careers, ethics, scientific strategy, interpersonal relationships, oral and written communication, graphics, and so forth. I realize that each young scientist has different talent sets, and thus try to help each individual improve all their skills. For example, we discuss appropriate and effective ways to network, how to turn potential competitors into collaborators, how to compete (if necessary) in a collegial way, etc. We also engage in open discussions about alternative career choices in addition to preparation for obtaining and succeeding in an academic career. I view my role as a mentor to primarily be a resource for the scholarly, academic and personal advancement of the careers of my trainees.*

***Writing**. In general, the PI does not write the research papers from his laboratory, but discusses content, organization and figures as the papers are planned and being written, edits to enhance the personal style of each author, and rewrites key parts if necessary. My goal is to train superb writers. Other laboratory members continually critique each other's manuscripts, grant proposals, research statements, posters, etc.*

***Scientific meetings and conferences.** Trainees attend and present her data at 2 or 3 scientific meetings each year, either locally, nationally or internationally. All trainees have presented their findings from this project at 3 scientific meetings in the past year. This provides not only the opportunity to receive feedback and critique on the project, but to network with researchers*

How were the results disseminated to communities of interest?

The PI has presented work from this project at 5 national and international meetings
The PI is scheduled to present this work at the 2018 World Parkinsons Congress, 2018 Federation of Neurogastroenterology and Motility meeting and 2018 Society for Neuroscience meeting

Both Dr. Timothy Sampson and Catherine Schretter have presented posters on this research at 2 meetings each

Reem Abdel-Haq and the PI have authored a review manuscript on the topic of this project that was recently accepted for publication after peer-review in the prestigious Journal of Experimental Medicine

- **What do you plan to do during the next reporting period to accomplish the goals?**

1) In the Year 2 of the Project, Dr. Keshavarzian's team at RUMC will continue vigorous patient and subject recruitment and sample collection (target for Year 2 for RUMC is 17 subjects). So far we have succeeded in hitting our enrollment target for human subjects

(16/16). 2) Microbiome sequencing and SCFA analysis will be done in batches. 3) Dr. Mazmanian's group will analyze motor symptoms, neuroinflammation and pathophysiology in the "humanized" mouse models following prebiotic treatment. 4) We will evaluate short chain fatty acid (SFCA) levels in the prebiotic treated mice. 5) Dr. Gradinaru's group will image brain tissues from these mice. 6) Drs. Mazmanian and Hamaker will finish the "optimized" prebiotic diets.

3. **IMPACT:** *Describe distinctive contributions, major accomplishments, innovations, successes, or any change in practice or behavior that has come about as a result of the project relative to:*

- **What was the impact on the development of the principal discipline(s) of the project?**

Rush University Medical Center site and Dr. Keshavarzian's team achieved the targeted new subject recruitment and enrollment (16/16) which is required for the success of the project. The animal studies at Caltech further corroborated the preliminary data for a role by SCFAs in motor symptoms in mice.

- **What was the impact on other disciplines?**

Nothing to report

- **What was the impact on technology transfer?**

Nothing to report

- **What was the impact on society beyond science and technology?**

Nothing to report

4. **CHANGES/PROBLEMS:**

- **Changes in approach and reasons for change**

Nothing to report

- **Actual or anticipated problems or delays and actions or plans to resolve them**

Nothing to report

Changes that had a significant impact on expenditures

Nothing to report

- **Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents**

Nothing to report

- **Significant changes in use or care of human subjects**

Nothing to report

- **Significant changes in use or care of vertebrate animals.**

Nothing to report

- **Significant changes in use of biohazards and/or select agents**

Nothing to report

5. **PRODUCTS:** *"Nothing to Report."*

- **Publications, conference papers, and presentations**

  Report only the major publication(s) resulting from the work under this award.

  - **Journal publications.**

Reem Abdel-Haq and the PI have authored a review manuscript on the topic of this project that was recently accepted for publication after peer-review in the Journal of Experimental Medicine

Drs. Sampson, Schretter and the PI have submitted an original manuscript based on this project that is currently in review at the prestigious journal Nature.

  - **Books or other non-periodical, one-time publications.**

Nothing to report

  - **Other publications, conference papers, and presentations.**

Nothing to report

Website(s) or other Internet site(s)

sarkis.caltech.edu

Technologies or techniques

Nothing to report

- **Inventions, patent applications, and/or licenses**

Nothing to report

- **Other Products**

Nothing to report

6. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

- **What individuals have worked on the project?**

NAME:	**Sarkis K. Mazmanian, PhD**
PROJECT ROLE:	PI, and Caltech Site PI
RESEARCHER IDENTIFIER:	
NEAREST PERSON MONTH WORKED:	1.20 Calendar Months
CONTRIBUTION TO PROJECT:	Dr. Mazmanian directs the overall project, as well as the Caltech site as it relates to his laboratory. He meets with the Caltech team weekly, as well as additional ad hoc meetings. He organizes and leads the monthly team call that includes the all groups involved at Caltech, Rush, Perdue, UCSD and U of Wisconsin.
FUNDING SUPPORT (If Applicable):	

NAME:	**Timothy R. Sampson, PhD**
PROJECT ROLE:	Postdoctoral Fellow, Investigator
RESEARCHER IDENTIFIER:	
NEAREST PERSON MONTH WORKED:	12.00 Calendar Months
CONTRIBUTION TO PROJECT:	Dr. Sampson leads all studies on the mouse motor testing, neuroinflammatory analysis, and pathophysiology studies.

FUNDING SUPPORT (If Applicable):	

NAME:	**Catherine E. Schretter, PhD**
PROJECT ROLE:	Graduate Student, Investigator
RESEARCHER IDENTIFIER:	
NEAREST PERSON MONTH WORKED:	12.00 Calendar Months
CONTRIBUTION TO PROJECT:	Dr. Schretter leads all studies on the prebiotic development and testing.
FUNDING SUPPORT (If Applicable):	

NAME:	**Livia Hecke Morais, PhD**
PROJECT ROLE:	Postdoctoral Fellow, Investigator
RESEARCHER IDENTIFIER:	
NEAREST PERSON MONTH WORKED:	12.00 Calendar Months
CONTRIBUTION TO PROJECT:	Dr. Morais leads all studies on the humanized mice.
FUNDING SUPPORT (If Applicable):	

NAME:	**Reem Abdel-Haq**
PROJECT ROLE:	Graduate Student, Investigator
RESEARCHER IDENTIFIER:	
NEAREST PERSON MONTH WORKED:	4.00 Calendar Months
CONTRIBUTION TO PROJECT:	
FUNDING SUPPORT (If Applicable):	Reem is partially supporting by graduate student fellowship.

NAME:	**Yvette Garcia-Flores**
PROJECT ROLE:	Senior Technician
RESEARCHER IDENTIFIER:	
NEAREST PERSON MONTH WORKED:	4.00 Calendar Months
CONTRIBUTION TO PROJECT:	
FUNDING SUPPORT (If Applicable):	

- **Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?**

Nothing to report

- **What other organizations were involved as partners?**

Nothing to report

7. **SPECIAL REPORTING REQUIREMENTS**

- **COLLABORATIVE AWARDS:** *N/A*
- **QUAD CHARTS:** *If applicable, the Quad Chart (available on https://www.usamraa.army.mil) should be updated and submitted with attachments.*

8. **APPENDICES:** *N/A*